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Comparison of Fluconazole and Amphotericin B for Treatment of Experimental *Candida albicans* Endocarditis in Rabbits

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Amphotericin B (AmB) and fluconazole, administered intraperitoneally for 7 days, were compared in a rabbit model for *Candida albicans* endocarditis. When given early, AmB was more effective than fluconazole for reducing CFU counts in vegetations ($P < 0.01$) and kidneys. Forty-eight hours after the last dose, AmB was still detected in all vegetations whereas fluconazole was detected in only one case.

Candida endocarditis is being reported with increasing frequency because of current medical practices which allow *Candida albicans* to adhere to prosthetic materials (2). At present, amphotericin B (AmB) remains the standard therapy for invasive *Candida* infection, despite being poorly tolerated. Fluconazole, an antifungal triazole, is effective in vitro against the majority of *Candida* species and has acceptable toxicity (12). It has been used successfully to cure disseminated candidiasis in noncomparative trials and in small groups of patients (1, 10). The comparison of AmB and fluconazole in several animal models of *Candida* infection has yielded different results (7, 8, 16). The aim of this study was to compare the efficacies of AmB (alone or in combination with flucytosine) and fluconazole in a rabbit model of *C. albicans* endocarditis.

Female New Zealand White rabbits (CEGAV, Saint Mars d'Egrenne, France) weighing ≈2.5 kg were used. Ketamine hydrochloride (50 mg, Ketalar; Parke-Davis, Courbevoie, France) was administered intramuscularly for anesthesia. The rabbits were sacrificed by intracardiac injection of 100 mg of pentobarbital sodium (Rhone-Poulenc, Paris, France).

Sterile endocarditis was produced by transaortic valvular placement of a sterile polyethylene catheter (Becton Dickinson, Paris, France) by the method of Durack and Beeson (4). The catheter was left in place for the duration of the study. Endocarditis was induced 48 h after the catheterization, by injecting 3×10^7 CFU of *C. albicans* into the marginal ear vein.

A strain of *C. albicans* was provided by B. Pangon (Department of Microbiology, Centre Hospitalier de Versailles, France). Twenty-four hours before in vivo studies, organisms were inoculated into a yeast-nitrogen base broth, incubated overnight, washed twice, and resuspended in 0.85% NaCl. Confirmation of infecting inoculum was obtained by CFU counts of serial 10-fold dilutions of the *C. albicans* suspension grown in a yeast-potassium-glucose agar.

The susceptibility of this *C. albicans* strain was determined before and after treatment by using the broth macrodilution method for fluconazole and AmB (3, 5) and the Mycototal (Behring) method for flucytosine. Similar results were obtained with the standardized National Committee for Clinical Laboratory Standards method.

Fluconazole, supplied as a powder (Pfizer, Orsay, France), was diluted to a final concentration of 12.5 mg/ml in 10% (vol/vol) Cremaphor EL (Sigma Aldrich Chimie, Saint-Quentin, France) in 0.85% NaCl buffered with 0.2 M sodium phosphate (pH 7). Fluconazole (100 mg/kg of body weight per day) was administered intraperitoneally (i.p.) to the rabbits once daily. Previous reports have indicated that similar peak concentrations in serum were obtained when this agent was administered to rabbits either intravenously (i.v.) or i.p. (17). AmB (Squibb, Neuilly-sur-Seine, France) was diluted in a 2.5% sterile glucose solution at a final concentration of 3.5 mg/ml. Flucytosine (Roche, Neuilly-sur-Seine, France) was prepared according to the manufacturer's instructions, and the dosages, administered i.p., were 40 mg/kg every 12 h for early therapy studies and 40 mg/kg every 8 h for delayed-therapy studies.

Two studies were performed. In the first one, rabbits received, 72 h after inoculation, for a total of 7 days, (i) no treatment ($n = 8$) or daily i.p. injections of (ii) AmB (10 mg/kg; $n = 11$) or (iii) AmB (10 mg/kg) in combination with flucytosine (40 mg/kg every 8 h; $n = 4$). In the second study, therapy was initiated 24 h after inoculation and continued for a total of 7 days with one of the following regimens: (i) no drug ($n = 8$) or daily i.p. injections of (ii) fluconazole (100 mg/kg; $n = 8$), (iii) AmB (10 mg/kg; $n = 7$), or (iv) flucytosine alone (40 mg/kg every 12 h; $n = 5$). Eight additional rabbits were sacrificed before initiation of therapy 24 h after infection.

AmB pharmacokinetics was determined after i.p. administration of 5, 10, or 15 mg/kg/day for 7 days to four noninfected and six infected animals by high-performance liquid chromatography (HPLC) as described previously (3). Fluconazole concentrations in infected animals were measured by HPLC (15) in blood drawn 1 h after the i.p. injection on the sixth day of treatment ($n = 6$) and 48 h after the last injection ($n = 5$). The limits of detection were 0.2 and 0.5 μg/ml for AmB and fluconazole, respectively.

AmB and fluconazole levels were determined within the vegetations by HPLC. Vegetations were kept frozen at −80°C until analysis. The limits of detection were, respectively, 0.2 and 0.5 μg/g of tissue for AmB and fluconazole. Nephrotoxicity was assessed by serum creatinine measurement. In rabbits treated with flucytosine at 40 mg/kg every 12 h, polymorphonuclear leukocytes were counted by a Coulter STKS instrument (Coultronics, Hialeah, Fla.) at the time of sacrifice.

All animals were sacrificed at least 48 h after the last antifungal dose. Hearts were removed, and the position of the indwelling catheter was verified. Vegetations were pooled,

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weighed, and homogenized in 1 ml of sterile normal saline, washed twice, serially diluted, and quantitatively cultured in yeast-potassium-glucose agar at 37°C for 24 h. In addition, a fragment of kidney was removed and cultured in the same manner. Results are expressed as $\log_{10}$ CFU/g of vegetation or organ. For statistical comparisons, vegetations yielding negative cultures were considered to contain ≤ 2 $\log_{10}$ CFU/g (i.e., the limit of sensitivity) on the basis of the average vegetation weight in this model. Differences in intravegetation *C. albicans* densities between treatment groups were compared by Student's *t* test for two groups and the Sheffe's test for more than two groups, when allowed by analysis of variance. Comparison of percentages was determined by χ^2 test. A P value of <0.05 was considered to be statistically significant.

RESULTS

MICs of AmB were 0.25 μg/ml before therapy. Values for susceptibility to flucytosine and fluconazole before and after therapy were ≤0.78 and 6 μg/ml, respectively. Serum AmB concentrations are given in Table 1. In the 10-mg/kg AmB group, the nephrotoxicity was mild but statistically significant (creatinine levels: mean ± standard deviation [SD], 68 ± 14 and 124 ± 40 μmol/liter on days 1 and 7, respectively; $n = 3$, $P < 0.05$). AmB at 15 mg/kg caused excessive mortality due to nephrotoxicity (serum creatinine level: mean ± SD, 292 ± 102 μmol/liter). No nephrotoxicity was observed in the 5-mg/kg group. The mean ± SD serum fluconazole concentration was 82.3 ± 20 μg/ml ($n = 6$), 1 h after i.p. administration of 100 mg/kg/day, and 7.8 ± 3.2 μg/ml ($n = 5$), 48 h after the last injection. The neutrophil blood count (mean ± SD) was $5.122 \times 10^9 \pm 1.9 \times 10^9$/liter at the end of therapy with flucytosine in three infected rabbits. AmB in combination with flucytosine was highly toxic; all treated animals died before the sixth day of therapy. No renal abnormality was detected. The trough concentration of AmB (mean ± SD) was 0.58 ± 0.49 μg/ml ($n = 3$) on the fifth day of combined therapy.

When therapy was initiated 72 h after infection, AmB failed to significantly reduce the fungal load in vegetations (5.08 ± 1.36 $\log_{10}$ CFU/g) compared with controls (6.11 ± 1.24 $\log_{10}$ CFU/g) at the time of sacrifice. The efficacy of AmB combined with flucytosine could not be evaluated.

When therapy was initiated 24 h after infection (Table 2), rabbits treated with AmB (10 mg/kg/day) had significantly reduced cardiac fungal loads compared with untreated controls ($P < 0.001$) and with animals treated with fluconazole (100 mg/kg/day) ($P < 0.01$) or with flucytosine (40 mg/kg every 12 h) ($P < 0.01$). Fluconazole and flucytosine were not effective for reducing fungal load in vegetations.

C. albicans counts in the kidney are shown in Table 2. All five untreated rabbits sacrificed 24 h after infection had positive cultures. Cultures were also positive in seven of nine untreated rabbits sacrificed at the assigned time, one of seven in the AmB group ($P < 0.05$) and six of seven animals in the fluconazole group.

Forty-eight hours after the last dose, no fluconazole was detected in the vegetations in five of six animals, whereas AmB was detected in the vegetations of all six animals studied (Table 3).

In this study, AmB was administered i.p. for several reasons: the concentrations in the blood obtained were within the range reported for patients treated with AmB (6), they remained stable between injections, and they were detectable 72 h after the last dose. Administered 24 h after the challenge, only Amb was able to significantly decrease the vegetation fungal load. However, the same regimen of AmB, administered later after

TABLE 1. Serum AmB concentrations after once-daily i.p. administration to noninfected animals

AmB unitary dosage (mg/kg/day)	AmB concentration measured (mean ± SD; μg/ml)											
	After first dose (day 1)						After last dose (day 7)					
	1 h	2 h	4 h	8 h	12 h	24 h	1 h	2 h	4 h	8 h	12 h	24 h	72 h
5[a]	0.21 ± 0.06	0.24 ± 0.05	0.21 ± 0.08	0.20 ± 0.09	0.30 ± 0.10	0.30 ± 0.20	0.41 ± 0.11	0.35 ± 0.12	0.35 ± 0.08	0.33 ± 0.07	0.28 ± 0.10	0.25 ± 0.05	ND[b]
10[c]	0.26 ± 0.14	0.54 ± 0.02	0.51 ± 0.15	0.61 ± 0.11	0.50 ± 0.20	0.67 ± 0.14	0.54 ± 0.35	0.63 ± 0.4	0.66 ± 0.45	0.73 ± 0.30	ND	0.66 ± 0.23	0.43 ± 0.3
15[d]	0.45 ± 0.40	1.16 ± 1.13	0.56 ± 0.27	0.66 ± 0.37	0.65 ± 0.30	0.71 ± 0.3	1.45 ± 0.32	1.27 ± 0.40	1.31 ± 0.3	1.20 ± 0.26	1.10 ± 0.39	1.14 ± 0.44	0.70 ± 0.43

[a] $n = 4$ on days 1 and 7.
[b] ND, not done.
[c] $n = 3$ on days 1 and 7.
[d] $n = 4$ on day 1, and $n = 2$ on day 7.

TABLE 2. Effect of treatment initiated 24 h postinfection on fungal load measured in cardiac vegetations and kidneys 48 h after administration of the last dose[a]

Regimen	$\log_{10}$ CFU/g (mean ± SD) (no. of rabbits)		No. of rabbits with negative kidney cultures/total
	Vegetation	Kidney	
Before initiation of therapy (baseline fungal load)	4.20 ± 1.16 (8)	3.16 ± 0.25 (5)	0/5
No treatment	5.77 ± 0.77 (8)	2.59 ± 0.41 (7)	2/9
AmB (10 mg/kg/day)	3.24 ± 1.74[b,c] (7)	1.60 (1)	6/7[d]
Fluconazole (100 mg/kg/day)	4.81 ± 0.71 (8)	2.05 ± 0.35 (6)	1/7
Flucytosine (40 mg/kg every 12 h)	5.00 ± 0.98 (5)		

[a] Animals were treated for 7 days.
[b] $P < 0.001$ compared with untreated group.
[c] $P < 0.01$ compared with group treated with fluconazole or flucytosine.
[d] $P < 0.05$ compared with untreated group.

infection (72 h), failed to significantly decrease the fungal load compared with the case for the control animals. These results show, as previously reported (16), that the relationship between the time of therapy initiation and efficacy is an important factor. The increase in CFU counts with time within the infected organ and the short duration of AmB therapy may explain the failure of delayed antifungal therapy. Witt and Bayer (17) reported comparable AmB failure in a similar model, when the drug was administered 60 h after challenge. To the best of our knowledge, such doses of fluconazole and AmB had not been compared in this model. Witt and Bayer (17) compared the efficacy of fluconazole (50 mg/kg/day, i.p.) with that of AmB (1 mg/kg/day, i.v.), administered 24 h after *Candida* challenge, and also found that AmB was more effective than fluconazole in decreasing the vegetation fungal load after 8 days of therapy. However, the dose of fluconazole was low and could have accounted for its failure. When compared with untreated controls, a higher dose (100 mg/kg/day) of fluconazole was still ineffective after 6 days of therapy but became effective after 14 days of treatment. In another report (9), fluconazole therapy initiated 72 h after infection and continued for 14 days was more effective than AmB in a rabbit *C. albicans* endocarditis model, but the AmB dose was low (3 mg/kg/day, i.p.). Finally, optimal doses of both antifungal agents were compared directly in a rabbit model of non-*C. albicans* endocarditis (18): AmB was more rapidly fungicidal than fluconazole against *Candida tropicalis* but equally effective after 11 days of therapy against *Candida parapsilosis*. The in vivo efficacy was attributed to differences in strain susceptibility.

In our study, fluconazole failure cannot be explained by strain resistance or low serum drug concentrations. No resistance appeared, mean peak concentrations were similar to those reported previously in rabbits (17), and the trough levels measured 48 h after the last once-daily administration were in the range of the peak concentrations observed in humans after a daily dose of 400 mg (14). The combination of AmB with flucytosine caused unexplained excessive mortality. Similar mortality rates had been reported previously by Sande et al. (13) and could be species related.

Kidney culture results should be interpreted with caution. In our study, kidneys from 20% of the untreated animals remained sterile at the time of sacrifice. In a disseminated *Candida* infection model, Filler et al. (7) reported that after 10 days of therapy, 20% of animals had infected kidneys in the AmB group (1 mg/kg/day, i.v.) compared with 70% in the fluconazole group (80 mg/kg/day, i.v.), findings which are in agreement with our results. Determination of antifungal agent concentration within vegetations has not been studied extensively (11). In our study, the very short time of exposure of the vegetations (a few seconds) to the washing saline solution probably prevented solubilization of the drugs. Since fluconazole levels were determined late after the last dose (48 h), we cannot exclude the possibility that fluconazole was transitorily concentrated in the vegetation at the time of the peak level of the drug in serum.

In a reproducible model of *Candida* endocarditis which approaches the human disease and at drug concentrations observed in humans, AmB was more effective than fluconazole in reducing vegetation fungal load and maintaining sterile renal tissue at the initial phase of therapy. Different modalities for treatment, such as longer duration of therapy, should now be evaluated in this model.

TABLE 3. Drug concentrations in infected vegetations and blood 48 h after the end of therapy, started 24 h postinfection[a]

Regimen (i.p.)	Drug concentration (mean ± SD) (*n*)	
	Vegetation (μg/g)	Blood (μg/ml)
Fluconazole (100 mg/kg/day)	28[b] (1)	7.8 ± 3.2 (5)
AmB (10 mg/kg/day)	1.98 ± 0.1 (6)	ND[c]

[a] Animals were treated for 7 days.
[b] Fluconazole was detectable in only one of five treated animals.
[c] ND, not done.

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